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外泌体在消化系统肿瘤早期诊断中的进展

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摘　要

目的：总结外泌体在消化系统肿瘤(Digestive system tumor)早期诊断中的应用研究进展，为后续相关研究提供思路与方法，便于相关研究尽快转化为临床应用。方法：阅读国内外外泌体在消化系统肿瘤研究的相关文献，进行整理、总结并综述。结果：目前，消化系统肿瘤是当前肿瘤相关死亡常见的病因之一且发病年龄趋年轻化。外泌体(Exosomes)可在分子间通讯发挥巨大作用，对外泌体的检测可达到对消化系统肿瘤的早期诊断，这对于该类肿瘤患者的治疗及预后有重要意义。结论：外泌体检测在消化系统肿瘤早期诊断是可行的，相关研究方法与技术正在向临床应用转化。

关键词

外泌体，消化系统肿瘤，早期诊断，食管癌，胃癌，结直肠癌，肝癌

Advances in Exosomes in the Early Diagnosis of Digestive System Tumor

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Abstract

Objective: To summarize the research progress on the application of exosomes in the early diag-

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nosis of digestive system tumors, to provide ideas and methods for the subsequent related research, and to facilitate the translation of related research into clinical application as soon as possible. Methods: The literatures related to the study of exosomes in digestive system tumors at home and abroad were reviewed, organized, summarized and synthesized. Results: Currently, digestive system tumors are the most common cause of tumor-related deaths and the age of onset tends to be younger. Exosomes can play a huge role in intermolecular communication, detection of exosomes can achieve early diagnosis of digestive system tumors, which is important for the treatment and prognosis of patients with this type of tumor. Conclusions: Exosome detection is feasible in the early diagnosis of digestive system tumors, and the related research methods and techniques are being translated into clinical applications.

Keywords

Exosomes, Digestive System Tumors, Early Diagnosis, Esophageal Cancer, Gastric Cancer, Colorectal Cancer, Liver Cancer

1. 引言

消化系统肿瘤(Digestive system tumor)的发病率和死亡率呈现逐年上升趋势，全球约有 480 万胃肠道肿瘤新发病例和 340 万相关死亡病例，占全球癌症发病率的 26%，占所有癌症相关死亡的 35% [1]。癌症的早期诊断可很大程度地降低癌症的死亡率，延长患者的生存期甚至治愈[1] [2]。外泌体(Exosomes)是细胞间通讯的重要载体，是细胞分泌的囊泡，包含蛋白质、miRNA、mRNA 等。外泌体为实现癌症的早期诊断提供了一种新的方法，通过液体活检技术实现对肿瘤的早期诊断是医学史上的一大突破[3]。本文就外泌体检测在消化系统肿瘤早期诊断的进展进行了综述，有助于进一步探讨外泌体检测在消化系统肿瘤早期诊断的可行性。

2. 食管癌

2.1. 流行病学与现有诊断方法

相关数据表明：食管癌(Esophageal Cancer, EC)每年增加了 57 万例，死亡 50.8 例[4]。食管癌的早期临床体征并不明显，这导致食管癌在晚期才能得到诊断，从而导致预后不佳。除了常见的影像学检查，食管癌的诊断主要依靠于消化内镜，虽然近年来消化内镜得到了蓬勃发展，可在较早期发现食管黏膜的病变，从而在一定程度上减轻了晚期食管癌带来的严重后果[5] [6]，但由于其为有创检查，且只有在食管发生了形态学和病理学上的改变时，方能得到确切的诊断，故其不是食管癌早期诊断的最佳选择。食管癌的肿瘤标志物在临床上被广泛应用，但其突出的缺点是特异性低。因此，我们迫切需要开发出一种特异性高且灵敏性高的新型标志物。

2.2. 外泌体在食管癌早期诊断进展

外泌体检测为食管癌的早期诊断提供了一种切实可行的方法，其将会成为实现食管癌早期诊断的里程碑。

2.2.1. 外泌体 microRNA 在食管癌早期诊断中的进展

Youhei Tanaka 等人发现在食管鳞状细胞癌(Esophageal squamous cell carcinoma, ESCC)患者血清中 miR-21 的表达水平较高，这表明 miR-21 可能是 ESCC 初始诊断的生物标志物，并证明血清外泌体 miR-21 与年龄、晚期肿瘤(T)分类、阳性淋巴结状态和转移相关[7]。miR-451a 可以削弱抗肿瘤免疫，促进肿瘤进展，其机制是 YWHAE 选择性地将 miR-451a 分选到外泌体中，因此我们可以通过对 miR-451a 表达量的检测来实现对食管癌的早期诊断[8]。MeiLing Xu 等人检测到 lncRNA LINC01711 在 ESCC 组织中高表达，认为其与预后不良相关，这是因为外泌体 LINC01711 可通过上调 FSCN1、下调 miR-326 促进食管癌细胞的增殖、迁移和侵袭，从而促进食管癌的发生发展[9]。

2.2.2. 其他外泌体在食管癌早期诊断中的进展

一系列实验证明：对于食管癌的诊断来说，细胞间粘附分子-1 (CD54)是极具潜力的生物标志物，可用于食管癌早期诊断的深入研究[10]。Chuanfeng Zhang 等人通过超快隔离系统(EXODUS)对放化疗期间的外泌体含量进行检测从而达到对食管癌治疗效果的评估及对复发监测[11]。除此之外，血清 let-7a 和 IL-6 水平的早期变化也可用作预测食管癌患者放疗反应的生物标志[12]。癌症相关成纤维细胞(CAF)衍生的外泌体蛋白也可用于评估预后及疗效[13]。Dongdong Yan 等人的研究首次证明：外泌体 M6PR 和 EphB4 在食管癌中起重要作用，尤其是在肿瘤血管生成和恶性肿瘤的发生发展，并且证实血清 M6PR 是食管癌患者的新型预后标志物[14]。

3. 胃癌

3.1. 流行病学与现有诊断方法

胃癌(Gastric cancer)是常见的恶性肿瘤之一，尤其在肿瘤晚期，其中位生存率低[15]。因此，实现早期诊断和预防对胃癌病人的治疗和预后有着重要的意义。目前临床上对胃癌早期诊疗的主流方式是胃镜[16]，但因其是有创检查，且只有在出现病变的时候才能做到早期发现，在某种程度上限制了其对于胃癌的早期诊断的应用价值。而临床上最常使用的生物标志物又普遍面临特异性相对较低的问题，因此，临床上常采取多种标志物联合的形式以尽可能早地实现对胃癌的诊断[17]-[20]。

3.2. 外泌体在胃癌早期诊断进展

外泌体在胃癌的早期诊断的相关研究这几年也大有进展。

3.2.1. 外泌体 microRNA 在胃癌早期诊断中的进展

Zebo Huang 等人的实验结果表明：胃癌患者血清中 6 种 miRNAs (miR10b-5p; miR132-3p; miR185-5p; miR195-5p; miR20a-3p; miR296-5p)的表达水平均明显高于 NCs [4]。Shibao Li 等人的 qRT-PCR 结果显示，胃癌组与健康对照组相比，lnc-GNAQ-6:1 的表达明显降低。ROC 曲线下面积为 0.732，高于 CEA、CA19-9 和 CA72-4 的诊断准确率[21]。而 lnc-GNAQ-6:1 的表达水平与性别、年龄、肿瘤转移、血清癌胚抗原(CEA)、碳水化合物抗原 19-9 (CA19-9)和碳水化合物抗原 72-4 (CA72-4)无关[21]。Yingying Sun 等人发现早期胃癌患者血清样品中 ITIH4 水平明显高于晚期胃癌或肝细胞癌患者血清样品中 ITIH4 水平[22]。Guodian Zheng 等人的研究显示：外泌体 miR-590-5p 对胃癌的早期检测和预测具有潜在的实用价值且可以通过积极调控胃癌的恶性潜能，胃癌患者的血清外泌体 miR-590-5p 水平降低[23]。除此之外，他们还发现外泌体 miR-590-5p 表达与年龄、性别、肿瘤大小、肿瘤部位、静脉浸润、细胞分化、淋巴结转移、幽门螺杆菌、her-2 表达水平等临床病理特征无显著相关性(均为 $p > 0.05$) [23]。Haoying Wang 等人的研究结果表明：胃癌中的 circ-RanGAP1、肝细胞癌中的 circUHRF1 和结直肠癌中的 circFMN2 分别通过外泌体

miR-877-3p、miR-449c-5p 和 miR-1182 调控肿瘤的恶性行为[24]。

3.2.2. 其他外泌体在胃癌早期诊断中的进展

Qiying Song 等人在其研究中发现循环外泌体 lncRNA-GC1 被确定为无病和总生存率的独立预后预测因子[25]。XiaoHuan Tang 等人综述了非编码 RNA (ncRNA)在近几年的相关研究，认为 ncRNA 是胃癌诊断、预后、甚至是治疗的有效生物标志物[26]。

4. 结直肠癌

4.1. 流行病学与现有诊断方法

作为世界上最常见的恶性肿瘤之一，虽然结直肠癌(colorectal cancer, CRC)发病率和死亡率均有下降趋势，但其发病和死亡人数仍处于较高水平[27]。

由于结直肠癌早期的症状体征不明显且不典型，故当前主要通过内镜[28] [29]、CT、MRI 及肿瘤标志物等来实现对结直肠癌的早期检测，大便检测也是当前的一个热门检测方向[30] [31]。但是目前没有直接的研究表明哪种诊断方式更优，这几种方法或多或少地存在一定的局限性[32]。有研究通过对结肠镜检查的荟萃分析发现：腺瘤的漏诊率为 26%，晚期腺瘤的漏诊率为 9%，锯齿状息肉的漏检率为 27%，近端晚期腺瘤、锯齿状息肉、扁平腺瘤和结直肠癌高风险患者漏诊率较高，分别为 14%、27%、34%和 33% [29]。增强 CT 也因为其图像清晰度高，扫描速度快，能更有效地判断肿瘤的位置、大小和侵袭程度和清楚地显示远处器官的转移而被广泛应用于临床[33]，但增强 CT 除了其本身的放射性[34]和检查费用较高外，还有研究显示，在对直肠癌的诊断中，增强 CT 的敏感性、特异性和准确性也分别只有 73.08%、78.85%和 74.52% [35]。

4.2. 外泌体在结直肠癌早期诊断进展

4.2.1. 外泌体 microRNA 在结直肠癌早期诊断中的进展

Li Min 等人认为：小细胞外囊泡(small extracellular vesicles, sEVs)衍生的 miRNA 作为一种早期检测 CRC 的具有前景的生物标志物[36]。Yawen Wu 等人的研究表明：外泌体 miR-1470 在结直肠癌患者中呈高表达，不仅如此，miR-1470 的表达量还与年龄、转移和 HDL 含量相关[37]。除了应用于结直肠癌的早期诊断，外泌体在指导结直肠癌的治疗方面也表现出了较大的作用，比如：Dong Wang 等人的研究表明：外泌体 miR-25-3p、miR-130b-3p 和 miR-425-5p 的血清水平与 CRC 的进展和转移相关[38]。Gaofeng Liang 等人的实验也表明 miR-21 可调节 5-FU 在结直肠癌的耐药性[39]。

4.2.2. 其他外泌体在结直肠癌早期诊断中的进展

循环外泌体中 Alu RNA 的水平被认为与结直肠癌进展有关，因此其被认为是结直肠癌极具潜力的诊断标志物，主要机制是 Alu RNA 可激活 NLRP3 (富含亮氨酸的核苷酸结合域)炎症小体的内源性核[40]。作为外泌体检测常见的物质，Xiaoqian Yu 综述了近年来非编码 RNA (ncRNA)在结直肠癌中的相关研究，表明 ncRNA 在结直肠癌的早期诊断、治疗、转移及预后评估等存在重要价值[41]。Fakhria A. Al-Joufi 等人发现：CRC 患者的血清和血浆生长因子结合蛋白 2 (IGFBP2)水平更高，可作为 CRC 初步鉴定和发展的诊断工具[42]。Juan Li 等人综述了外泌体 circRNAs 在癌症诊断和治疗中的临床应用[43]。Ayuko Hoshino 等人介绍了细胞外囊泡和颗粒(extracellular vesicles and particles, EVPs)蛋白可作为癌症检测和确定癌症类型的可靠生物标志物[44]。

当然，不可置疑的是，未来一定还会有像粪便标记物[45]、表观遗传改变生物标记[46]等多样的方法用于结直肠癌的早期诊断。

5. 肝癌

5.1. 流行病学与现有诊断方法

根据美国癌症学会最新的统计数据，肝癌的 5 年相对生存率只有 22%，仍是生存率最低的癌症之一，尤其对于女性患者，肝癌的发病率仍在继续增加[27]。

早期肝癌没有典型的影像学表现，这就突出了综合评估在肝癌早期诊断的重要性。临床上常用的肝癌诊断方法主要有超声[47]，CT [47]-[49]，MRI [47]-[50]等[51] [52]，常用的生物标志物有 AFP [47]，CA19-9 [51]和 CEA [51]。其中，对于肝癌的早期筛查，临床主要应用超声和 AFP [50] [53]-[55]。超声因为其无创，价格低廉而成为最被推荐的诊断方法[50] [56] [57]，但是对于严重肥胖或肝萎缩的患者，尤其是在检查膈穹顶下的病变时，超声的局限性就表现了出来[50]，早期分化良好的 HCC (<1.5 cm)在 B 超上的典型表现不明显，除非是具有一定技术水平的诊断人员才不至于漏诊[50]。虽然超声检测任何阶段 HCC 的敏感性为 84% (95%置信区间[CI] 76%~92%)，但早期 HCC 的灵敏度仅为 47% (95%可信区间 33%~61%) [52]。AFP 虽然在临床上作为诊断肝癌的常见生物标志物，但因其在肝癌早期很少出现异常[48]且其敏感性只有 41%~64%，特异性 80%~94%，其诊断性有限[58]，常常会有漏诊[59]。因此，众多学者开始寻找同时兼具高敏感性、高特异性且低廉微创的肝癌早期诊断方法。外泌体作为近年来生物标志物检测的热门方向，其在肝癌早期诊断上也受到了极大的关注。

5.2. 外泌体在肝癌早期诊断进展

Xiaocui Wei 等人在其研究中表明：外泌体不仅可能作为肝癌的潜在生物标志物或治疗生物靶点，并且可能作为肝癌患者的药物载体和自我治疗因子[60]。Juan Wang 等人认为 EVs 可能是 HCC 分子诊断的有效生物标志物和肿瘤靶向治疗的新靶点[61]。Yi-Te Lee 等人也在其研究中介绍了 EVs 在早期 HCC 检测中的潜在应用[62]。

5.2.1. 外泌体 microRNA 在肝癌早期诊断中的进展

microRNA (miRNA)是外泌体中较早开始研究的物质[63] [64]。近年来，miRNA 相关研究成果层出不穷。对于甲胎蛋白不敏感的肝癌，Suchandrima Ghosh 等人介绍了外泌体里面的各种 miRNA 在早期肝癌中的变化趋势及其发挥的作用[65]。Andrei Sorop 等人在其综述中详细介绍了外泌体 miRNA 作为肝细胞癌诊断和治疗的一种生物标志物在其中所起的作用[66]。除了 miRNA，外泌体中越来越多的新的生物标志物得到了广大研究者的喜爱。

5.2.2. 其他外泌体在肝癌早期诊断中的进展

Yucel Aydin 等人的试验表明：glypican 3 (GPC3)的循环外泌体可以用作肝硬化患者 HCC 检测和治疗反应的生物标志物[67]。Mengtao Xing 等人在其综述中提到：包括针对肿瘤相关抗原(TAA)和外泌体的自身抗体的 HCC 的免疫诊断标志物来提供对 HCC 的早期检测[68]。Chunfeng Qu 等人将 AFP、DCP 这两种血清蛋白标记物与 cfDNA 改变相结合，以研究这种基于液体活检的检测方法(包括和 AFP、DCP、cfDNA)是否是筛查早期 HCC 的有效方法[69]。Lei Zhu 等人在其研究中发现：肝癌患者血浆外泌体中四种 tsRNA (tRNA-ValTAC-3、tRNAGlyTCC-5、tRNA-ValAAC-5 和 tRNA-GluCTC-5)的水平显著升高，表明血浆外泌体 tsRNA 可以作为一种新的诊断生物标志物[70]。Wei Liu 等人的研究证实：血浆 Hsp90α 是一种新的泛癌诊断生物标志物，尤其是 AFP 限制性肝癌患者的敏感性为 91.78%，特异性为 91.96% [71]。在众多科研工作者的努力下，相信兼具高敏感性、高特异性的外泌体会很快应用于早期肝癌的临床诊疗。

5.3. 其他标志物在肝癌早期诊断进展

当然，除了外泌体，也有相关研究发现：类似肝癌的中枢基因 CDK1、HMMR、PTTG1 和 TTK 也可能是肝癌早期诊断的又一可靠生物标志物[72]，这有待这一步的研究。

6. 总结与展望

消化系统肿瘤的早期诊断、无创诊断、精确诊断仍是当前肿瘤诊治的一项重大挑战，外泌体及相关研究的出现，加快了实现消化系统肿瘤早期诊断的进程。本文分别综述了外泌体在食管癌、胃癌、结直肠癌和肝癌早期诊断中的研究进展，证明外泌体在实现食管癌、胃癌、结直肠癌和肝癌早期诊断中的重要意义，为实现消化系统肿瘤在临床早期诊断提供理论依据和基础，并为后续相关研究提供思路与方向。

但是，外泌体在肿瘤诊治方面相关的研究还存在一定的局限性。首先，外泌体是一个相对来说比较大的概念，外泌体中包含蛋白质、microRNA、mRNA 等物质，不同种类的蛋白质、microRNA 和 mRNA 在不同肿瘤上呈现不同的表达，尤其是一种外泌体可在多种肿瘤上表达，同时，一种肿瘤上也存在多种外泌体的表达，这在很大程度上降低了外泌体在肿瘤早期诊断的特异性，也是当前外泌体相关研究转化为临床应用的局限。其次，当前对于外泌体获取、提纯及转化等没有统一的技术标准，不同方法提取的外泌体在纯度、浓度等方面可能存在差异。最重要的是，现阶段外泌体的相关研究基本还停留在实验室阶段且由于其设备、耗材较昂贵，缺少临床数据的支持。

因此，在后续的研究中，首先要尽量使外泌体的概念细化，精确到具体外泌体的研究，在实现早期诊断的同时兼顾精确诊断。除此之外，在众多的外泌体提取方法当中，业内需尽快统一相关标准，使用获益大、纯度高、成本低的提取方法，以便尽快推进相关研究的进展。相信在科研工作者的不断努力下，外泌体在肿瘤早期诊断，甚至是后续的肿瘤治疗及预后评估上，能尽快地走出实验室，走向临床，服务广大群众。

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